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Biological Control of Cocoa Pod Borer (*Conopomorpha cramerella* Snell) Using Egg Parasitoids of *Trichogrammatoidea bactrae fumata* in East Java

Dwi Suci Rahayu[1*)] and Endang Sulistyowati[1)]

[1)]Indonesian Coffee and Cocoa Research Institute, Jl. PB. Sudirman No. 90, Jember, Indonesia
[*)]Corresponding Author: ds.rahayu13@yahoo.com
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Abstract

A study on the potential of *Trichogrammatoidea bactrae fumata* (*Tbf*) parasitization in the field was conducted at an insecticide-free cocoa cultivation namely Treblasala Plantation, Banyuwangi, East Java. The research design used was a factorial randomized block design with three replications. The tested treatments consisted of parasitoid release intervals (once in 2 and 4 weeks) and the release population of 0 (control); 25,000 and 50,000 *Tbf* per hectare per release. Observations were made on number of detected eggs and parasitized eggs, and level of cocoa damage caused by cocoa pod borer (CPB) attacks. The results of this research showed that parasitization level of *T. bactrae fumata* on CPB eggs after 3 months of the release ranged from 29.81–42.79%. The parasitoid release of CPB eggs (*T. bactrae fumata*) in the field for a year showed that the parasitization level of CPB eggs in the field ranged from 17.78–29.72%. Nevertheless, during that year, there was no significant effect of parasitoid release on the suppression of yield loss due to CPB attacks in the field.

Keywords: *Conopomorpha cramerella*, cocoa, parasitoid, biological control, *Trichogrammatoidea bactrae fumata*

INTRODUCTION

Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen is a dangerous and harmful pest of cocoa cultivation found in Asia Pacific countries such as Indonesia, Malaysia, Philippines and Papua New Guinea. Currently, CPB pest attacks have spread to all areas of Indonesian cocoa cultivation with various attack intensity, which becomes a threat to the continuity of cocoa production. The percentage of CPB attacks to cocoa pods of 16–95% can cause a significant yield loss with a percentage ranging from 3–56% (Sulistyowati *et al.*, 2015).

To date, various CPB control techniques have been applied, including culturally technical controls of cropping (P), fertilization (P), frequent harvest (P), and sanitation (S) that are more well-known as P3S; biological control, pheromone use and chemical control by spraying insecticides. Based on the results of a survey, 90% of cocoa farmers still use chemical insecticides for CPB control. This is one of the factor Indonesian cocoa beans often claimed by cocoa bean-import countries such as Japan and United States due to the low quality and the content of pesticide residues (Sulistyowati *et al.*, 2015). Excessive use of chemical insecticides can also have a negative impact on either target pests, non-target insects or the environment. Therefore, pest control alternatives that are free of pesticide residues should be sought, one of which is by the utilization of egg parasitoids from *Tricho-grammatidae* family as biological control agents. The famous genera as egg parasitoids are *Trichogramma* and *Trichogrammatoidea.*

Utilization of egg parasitoids has long been done in the field of agriculture and forestry. According to Rachman (2006), *Trichogramma* is widely used to control insect pests that are economically harmful, such as *Ostrinia* sp. (Lepidoptera: Pyralidae); *Chilo* sp. (Lepidoptera: Pyralidae) and *Helicoverpa* sp. (Lepidoptera: Noctuidae). Several studies on *Trichogramma* identification and release have been widely undertaken. The identification results of *Trichogrammatidae* parasitoids from various habitats and hosts suggested that of 10 selected *Trichogrammatidae* populations, there was a species of *Trichogrammatoidea bactrae-bactrae* from soybean plants showing the highest parasitization level of 73.60% (Marwoto, 2010). The utilization of *Trichogramma armigera* to control pod borer insects can reduce soybean pod damage by 23.27% with the parasitization level of 37.77% (Ramlan, 2001).

Based on the exploration results of CPB-natural enemies in the cocoa cultivation in North Maluku and North Sulawesi, the egg parasitoids of *Trichogrammatoidea* spp were found with the level of parasitization ranged from 18.6–84.7% (Sulistyowati *et al.,* 2001). The identification results based on the *Trichogrammatoidea* genetalia genera morphology of male insects showed that there are two species of egg parasitoids namely *T. bactrae fumata* derived from Teling and Kumu (North Sulawesi) and *Trichogrammatoidea cojuangcoi* derived from Berambai (East Kalimantan). The parasitizing ability of an egg parasitoid during the lifetime of *T. bactrae fumata* derived from Teling, Kumu and *T. Cojuangcoi* was able to reach respectively 45.1; 61.6; and 22.7 *Tbf* with sex ratios between male and female parasitoids from Kumu and Teling of respectively 5:2 and 4:3 (Sulistyowati & Wiryadiputra, 2009). In Sabah Malaysia, the parasitization level of *Trichogrammatoidea bactrae fumata* (*Tbf*) to control CPB pests in cocoa could reach 63-66%. The parasitoid release of 75,000 *T. bactrae fumata* every three days was able to suppress the yield loss equal to the pyrethroid insecticide treatment of 24 times per year (Liau, 1991). The aim of this study was to investigate the potential of *T. bactrae fumata* to parasitize cocoa pod borer in the field.

MATERIALS AND METHODS

The research was undertaken at a private cocoa farm in Banyuwangi, East Java. Elevation at the research site was 276 m asl. with B type rainfall according to Schmidt & Ferguson. Cocoa plants were planted in 1992 (plant spacing of 3 m x 3 m) with coconut shaded.

Additionally, this research was prepared according to a factorial group randomized block design with three replications. The first factor was the parasitoid population of *T. bactrae fumata* (*Tbf*) from North Sulawesi consisting of three levels *i.e.* 0 *Tbf* (A1), 25,000 *Tbf* (A2), 50,000 *Tbf* (A3) per hectare per release. The second factor was the parasitoid release intervals consisting of two levels *i.e.* 2 weeks (B1) and 4 weeks (B2); so that it was obtained some treatment combinations namely A1B1; A1B2; A2B1; A2B2; A3B1; A3B2.

Preparation of *Tbf* was conducted in the Plant Protection Laboratory of Indonesian Coffee and Cocoa Research Institute. Mass culture of *Tbf* used alternative hosts in the form of pest egg sheds of *C. cephalonica.* Host insects of *Corcyra cephalonica* were cultured on mixed media in the form of corn starch and corn rice. The insect larvae cultivation box of *C. cephalonica* was 30 cm x 40 cm x 5 cm, containing mixed media in the form of corn starch and corn rice with a ratio of 1:2. The cultivation box was then covered with a 5 mm-thick plywood equipped with wire netting vents. The cylindrical nesting cage was made of cardboard with a diameter of 10 cm and a height of 25 cm, with the top and bottom of the nesting cage made of wire netting. The egg 'pias' made of 2 cm x 2 cm-sized *manila* paper can contain ± 2000-2500 eggs of *C. cephalonica.*

The release of *Tbf* was performed by hanging the 'pias' (sized 2 x 2 cm) containing *Corcyra cephalonica* eggs that had been parasitized by *Tbf* in a pipe with a diameter of 2.5–5 cm and a length of 10 cm. The pipe was suspended on the branches of cocoa plants and given glue that can prevent ant attacks. Observations were made on the number of found eggs and parasite eggs, and the parasitization level observed monthly for a year. Moreover, the level of cocoa pods damage due to CPB attacks was also studied *i.e.* starting from the early release to every two or four weeks of harvest round.

The observation of cocoa pod damage level due to CPB attacks was based on the percentage of sticky seeds expressed in three categories covering *low*, *moderate*, and *high* attack levels. Cocoa pods would be said to be at low attack level if the percentage of sticky seeds was <10%. Meanwhile, it tended to be at moderate and high attack levels if the percentages of sticky seeds obtained respectively 10-50% and >50%. Each criterion of pods damage was weighted *i.e.* healthy pods (Ht, weighted of 0), low attack (L, weighted of 1), moderate attack (M, weighted of 3), and high attack (H, weighted of 9). The intensity of CPB attacks was calculated based on the following formula:

$$AI = \frac{(0*Ht+1*L+3*M+9*H)}{9*\text{ Number of pods observed}}$$

$$IS = \frac{(I*L+3*M+9*H)}{\text{Number of pods observed}}$$

According to Wardani *et al.* (1997), the yield lost estimation is calculated using the regression equation as follows:

$$Y = - 0.0210 + 0.1005\ X$$

Description :
AI : Attack intensity
Ht : Number of healthy pods
L : Number of pods with low pest attack level
M : Number of pods with moderate pest attack level
H : Number of pods with high pest attack level
IS/X : Intensity score of pest attack (*damage intensity*)
Y : Yield loss

To determine the effects of the parasitoid population and release intervals of *Tbf* on parasitization levels, the percentage of CPB attacks and the percentage of yield loss caused by the CPB attacks on some treatment combinations were tested using ANOVA test. For further analysis, it was used further orthogonal polynomials.

RESULTS AND DISCUSSION

The observation results of CPB attack percentages prior to the parasitoid release of *Tbf* showed that CPB attacks in the research site ranged from 54.86–68.97% with the yield loss ranging from 6.83–9.08%. The complete data are listed in Table 1. At the beginning of the parasitoid release, there was no significant difference between the percentages of CPB attacks, yield loss, and the number of CPB inlets.

Based on the average data of yield loss (Figure 1) caused by CPB attacks on the experimental area, it appeared that at two months after the parasitoid release, the percentage of yield loss due to CPB attacks was still increasing. However, on the three-month observation after the parasitoid release, decreased yield loss began to occur, although it was not significantly different based on the variance analysis (Table 2). The decrease was thought to be the results of parasitoid release because the three month-aged pods at the time of parasitoid release began to ripen on the three-month observation, indicating that the CPB egg parasitization can lead to a decrease in CPB attacks. In addition, in the control area, there also occurred a decrease in yield loss. Apparently, this was caused by the migration of parasitoids from the area/plot of release treatments to the control plot/area. This can be seen from Figure 2 showing the parasitization on CPB eggs taken from the control area, which ranged from 5.95–18.6%.

According to Usyati (2003), parasitoid displacement in plants is mostly influenced by wind movement and flying ability. Parasitoid insects were able to make an average flight of 5-8 times/minute or even could increase to 10 times or more during quiet wind. The parasitization level of *Trichogramma pretiosum* egg parasitoids on *C. cephalonica* hosts in the field spreading opposite to the wind direction was 76.97%; while that spreading in the same direction with the wind was only 23.03%. The spread of insects is not always in the same direction with the wind because there is a more interesting factor for insects that is the presence of food sources located in the opposite direction of the wind (Rahman, 2006).

Based on the average data of yield loss caused by CPB attacks in the experimental area, it was found that at ten months since the first parasitoid release, the percentage of yield loss due to CPB decreased to 0.95% while in the control area, it decreased to 2.04%. In the subsequent observation, there was an increase in yield loss, in which the average percentage of yield loss on the 14-month observation since the first release was between 13.57% and 4.15% at the dose of 25,000 parasitoids with the release intervals of 2 and 4 weeks. At the dose of 50,000 parasitoids with the release intervals of 2 and 4 weeks, the average yield loss obtained 8.22% and 6.73%. Meanwhile, the average percentage of yield loss at the control ranged from 6.59% to 8.19%.

Based on the overall observation, the percentage of yield loss due to CPB attacks still tended to fluctuate. There was no apparent reduction in the percentage of yield loss. This was presumably because the parasitization level was still low. According to Rachman (2006), the low parasitization level can be caused by various factors such as biological, physical environment, and chemical factors. These factors influence parasitoid behaviours in the host discovery stage that affect the parasitization level of parasitoids. The biological, physical environment and chemical factors greatly affect parasitoids in locating hosts. Biological factors such as lifetime, fitness, generation duration, body size, etc. can affect parasitoid activities in the field.

Table 1. Preliminary observations of CPB attacks, CPB yield loss, and number of CPB entry holes

Parasitoid population	Release period (Weeks)	CPB attacks (%)	Yield loss due to CPB (%)	Entry holes number (per treatment plot)
Control	2	68.97	8.39	12.67
	4	54.86	8.72	6.67
25,000	2	66.82	6.83	13.00
	4	57.60	6.91	8.33
50,000	2	60.40	6.89	10.33
	4	56.97	9.08	13.00

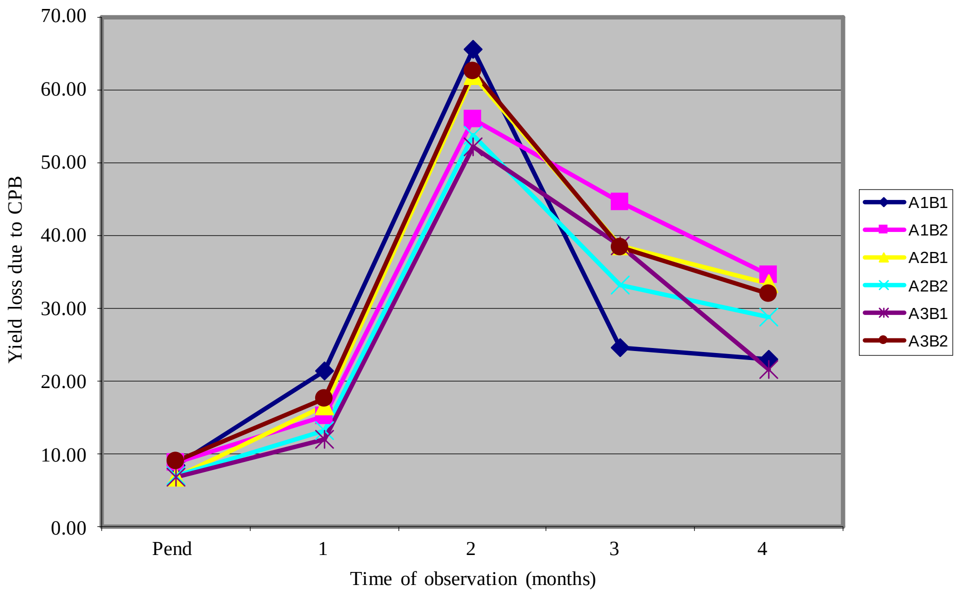

Figure 1. Average yield loss due to CPB attacks before and after parasitoid release of *T. bactrae fumata*

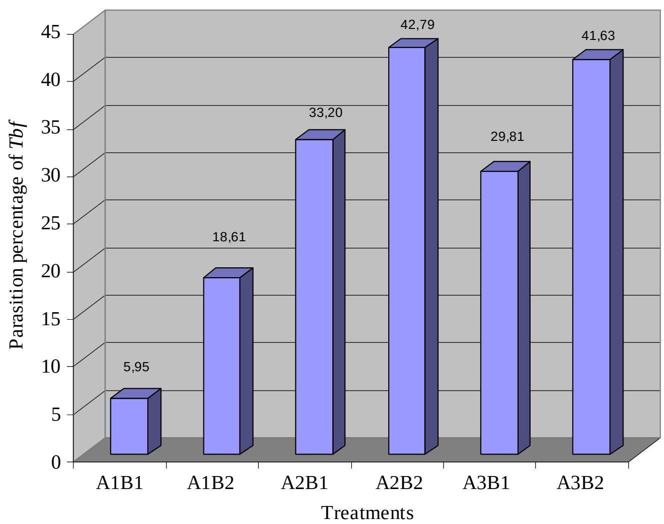

Figure 2. Parasitization levels of *T. bactrae fumata* on CPB eggs in the field after three month parasitoid release

Table 2. Variance analysis of effects of parasitoid population dose, release interval and the interaction between both parasitoid population dose and release interval at three months after the release

Sources of diversity	df	MS-parasitization	MS attack	MS-yield loss
Dose of parasitoid (a)	2	250.06 ns	555.17 ns	207.72 ns
Release interval (b)	1	1586.72 ns	5.56 ns	272.22 ns
a x b	2	581.72 ns	85.72 ns	42.39 ns
Error	10	246.12	143.63	125.79

Notes: ns = not significant, MS = Mean square.

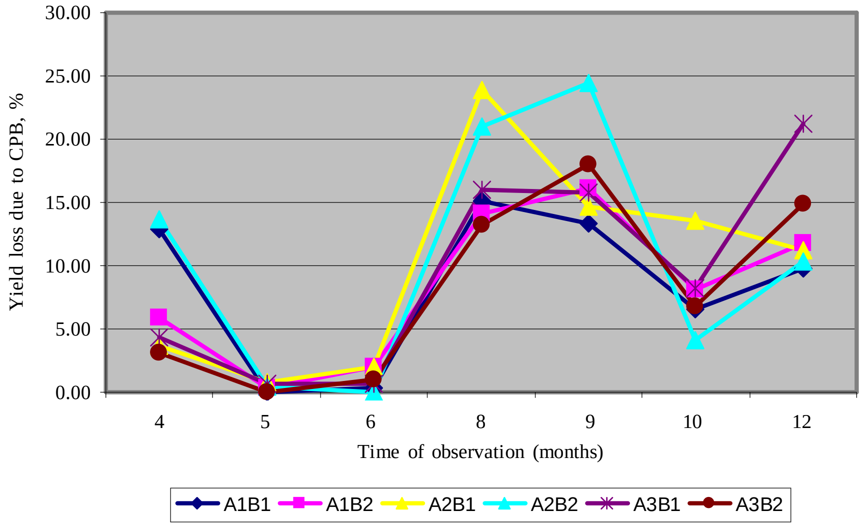

Figure 3. Average yield loss percentage due to CPB after parasitoid release of *T. bactrae fumata*

Physical environment factors such as light, dew, moisture, temperature, colour and shape of plants can be used as signals for parasitoids finding their host habitat.

The inability of natural enemies in controlling pest population in an agroecosystem can be caused by various things including the low population density of natural enemies that is unable to provide a rapid numerical response in order to compensate for the increase in pest populations. The low pest population density may be caused by human actions of using pesticides frequently. Another reason is weather changes that are more favourable to the development of pest populations and disadvantageous for the development of natural enemy population (Untung, 2006).

Based on the observation results of parasitization levels, it was apparent that there was no significant difference between the release dose treatments of 50,000 and 25,000 parasitoids as well as between the parasitoid release intervals of 2 weeks and 4 weeks. However, all those treatments were significantly different from the control, with 0% parasitization. At the dose of 25,000 parasitoids with the release intervals of 2 and 4 weeks, the parasitization levels of *T. bactrae fumata* were between 25.0% and 29.72%. Meanwhile, at the dose of 50,000 parasitoids, the parasitization levels were between 17.78% and 25.0%. Compared to the three-months observation after the release, these percentages of parasitization levels experienced a decrease. This was allegedly due to the high population of black ants in the release area, resulting in the death of *T. bactrae fumata* pupas due to being preyed by black ants, *Dolichoderus thoraxicus* which is also the natural enemy of CPB. In addition, the decrease in parasitization levels was estimated because *Trichogrammatoidea* parasitoids have a short life cycle so that the available time to search hosts was also reduced. According to Yuanita (2002), parasitoids of *Trichogrammatoidea* group take only 8 days to live. The life cycle of *Trichogrammatoidea* is strongly affected by temperature. At the temperature of 24^{O}C, *Trichogrammatoidea* needs 9 days to complete the life cycle while at the temperatures up to 32^{O}C, *Trichogrammatoidea* can complete its growth for 7 days.

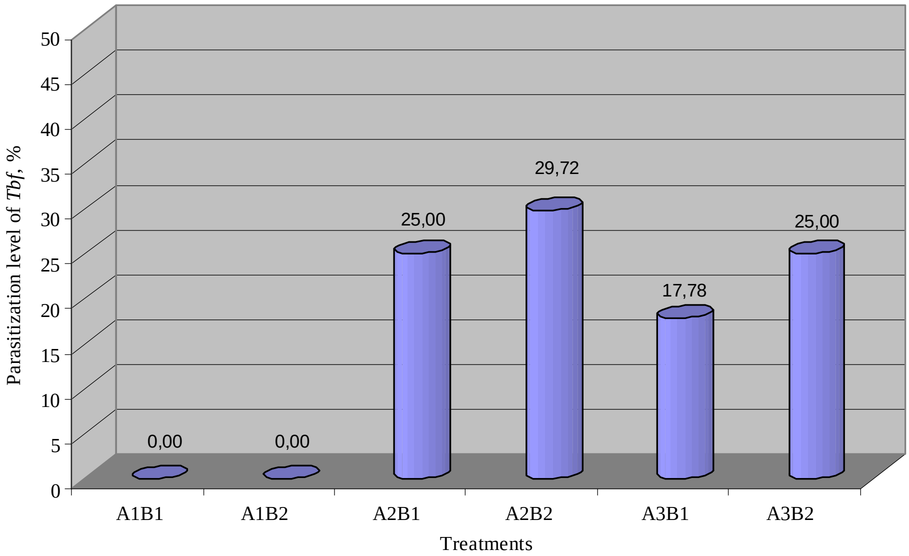

Figure 4. Parasitization levels of *T. bactrae fumata* on CPB eggs in the field after a year of release

Decreased levels of parasitization strongly depend on parasitoid and host interactions. Parasitoid response changes can be driven by parasitoid behaviour changes in host reception at different temperatures, reproductive ability, and female parasitoid lifetime. The changes can also occur due to field conditions that are continuously changing. Fluctuations in rainfall and light intensity in the field can affect parasitoid activities and behaviours which then also affect the level of parasitization. Moreover, temperature changes may also affect the activities and behaviours of parasitoids *e.g.* in the handling and search rate of hosts. Increasing the temperature to a certain level can decrease the host handling time. The level of parasitization will increase in line with the increased temperature to a certain extent because the parasitization interval becomes shorter. Chemical factors such as chemical compounds released by plants can be a sign of parasitoids in finding hosts (Wang & Ferro, 1998).

Land conditions such as size, shape, density and morphology of plants (leaf bone, hair, tissue form) also affect parasitoid behaviours in finding hosts and its success in parasitization (Bigler *et al.*, 2007). Physical diversity of a habitat also affects the parasitoid response to host density. This is related to the fact that a habitat is a refuge and alternative food source for parasitoids. Changes in plant size and shape may also affect the search rate and survival of parasitoids in the plant (Rachman, 2006).

The effectiveness of parasitoid release is influenced by pest population density, parasitoid population, and suitable environment (microclimate). To make effective parasitoid release, several things required to consider are the dynamics of pest population and the timing of egg laying in relation to the plant phenology, environmental factors that lead parasitoids to be persistent and active to locate hosts, parasitoid speeds to spread and locate hosts, right timing for parasitoid release, distance between the release points, the number of released parasitoids and the negative impacts of the use of pesticides from elsewhere that can spread to the release areas (Marwoto, 2010).

In Sabah Malaysia, the parasitization level of *T. bactrae fumata* to control CPB attacks in cocoa can reach 63-86%. The release of 75,000 *T. bactrae fumata* every three days was able to suppress the same yield loss with the pyrethroid insecticide treatment of 24 times/year (Liau, 1991). Based on the results of this research, the parasitoid release of CPB eggs (*T. bactrae fumata*) in cocoa farms especially in East Java for a year had still no significant influence on the suppression of yield loss due to CPB in the field. Therefore, further research is required by increasing the parasitoid population dose of *T. bactrae fumata* and shortening the release interval with longer periods of time.

CONCLUSIONS

Results of three-month observations after the parasitoid release in the field suggest that the parasitization levels of *T. bactrae fumata* on CPB eggs ranged from 29.81–42.79%; 12.66–36.84% higher than the control. The parasitoid release of CPB eggs (*T. bactrae fumata*) in the field for a year suggests that the parasitization level of CPB eggs in the field ranged from 17.78–29.72%. There was no significant effect of parasitoid release on the suppression of yield loss due to CPB attacks in the field.

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